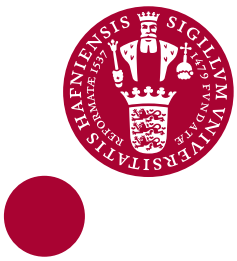

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Short Communication

Comparative methane estimation from cattle based on total CO_2 production using different techniques

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ABSTRACT

The objective of this study was to compare the precision of CH_4 estimates using calculated $CO_{2\ (HP)}$ by the CO_2 method (CO_2T) and measured CO_2 in the respiration chamber (CO_2R). The CO_2R and CO_2T study was conducted as a 3 × 3 Latin square design where 3 Dexter heifers were allocated to metabolic cages for 3 periods. Each period consisted of 2 weeks of adaptation followed by 1 week of measurement with the CO_2R and CO_2T. The average body weight of the heifer was 226 ± 11 kg (means ± SD). They were fed a total mixed ration, twice daily, with 1 of 3 supplements: wheat (W), molasses (M), or molasses mixed with sodium bicarbonate (Mbic). The dry mater intake (DMI; kg/day) was significantly greater ($P < 0.001$) in the metabolic cage compared with that in the respiration chamber. The daily CH_4 (L/day) emission was strongly correlated ($r = 0.78$) between CO_2T and CO_2R. The daily CH_4 (L/kg DMI) emission by the CO_2T was in the same magnitude as by the CO_2R. The measured CO_2 (L/day) production in the respiration chamber was not different ($P = 0.39$) from the calculated CO_2 production using the CO_2T. This result concludes a reasonable accuracy and precision of CH_4 estimation by the CO_2T compared with the CO_2R.

1. Introduction

Methane (CH_4) is a byproduct of rumen fermentation produced by methanogenic archaea. Methanogens use hydrogen (H_2) in the rumen to produce CH_4. Thus, they keep H_2 pressure low which favors anaerobic fermentation of ingested materials. Cattle are some of the main contributors of anthropogenic CH_4 gas emissions to the atmosphere (Gerber et al., 2013). This particular greenhouse gas has received a great deal of attention in the recent years not only because of its involvement in global warming processes leading to climate change, but also because it represents a loss of energy from the animals. Typically, methane emissions are about 2% to 12% of the gross energy intake depending on e.g., roughage-to-concentrate ratio in the feed, carbohydrate composition and use of supplements and additives (Johnson and Johnson, 1995). Enteric CH_4 production is a process very closely related to the composition of the volatile fatty acids produced in the rumen (Johnson and Johnson, 1995). The primary substrate for methanogenesis is H_2 that is generated during fermentation of plant cell wall carbohydrates. The products of this fermentation are primarily acetate and butyrate (Moss et al., 2000). Fermentation of starch and other non-structural carbohydrates favor propionate production. Propionate production is a competitive pathway for H_2 use in the rumen (Benchaar and Greathead, 2011). Unlike starch, fermentation of sugar by rumen microbes has been reported to increase methane production (Hindrichsen et al., 2004). Rumen microbial fermentation of sugar leads to a preferential production of butyrate at the expense of propionate (Friggens et al., 1998), hence, results higher methane production.

The respiration chamber was the only method for methane estimation from cattle for hundreds of years. Currently, several methods have been developed to estimate the actual emissions from livestock. They are based on different principles and have a wide range of optimal applicability (Storm et al., 2012). One of the methods with a wide applicability, the CO_2-method (CO_2T) is described by Madsen et al. (2010). The CO_2T uses the total CO_2 production from the animal as a marker for CH_4 estimation. The

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hypothesis of this study was that the precision of the CH_4 estimates by the CO_2T (using calculated total CO_2) would be comparable with a reference method (CO_2R; using measured total CO_2). Therefore, the present study was designed to compare the precision of CH_4 estimates between the CO_2T and CO_2R technique.

2. Materials and methods

2.1. Experimental design, animals and feeding

This present study was conducted with a 3 × 3 Latin square design where 3 Dexter heifers were allocated to balance cages for 3 periods consisting of 2 weeks of adaptation followed by 1 week of measurement. The animals were weighed at the start and end of the experiment. The average body weight (BW) of the heifers was 226 ± 11 kg (means ± SD) and the average dry matter intake (DMI) was 5.1 ± 0.3 kg/day (means ± SD) throughout the entire experiment. The animals were fed twice daily with a total mixed ration (TMR) made up (on DM basis) of 49% grass-clover silage, 14% soybean meal along with 35% of 1 of 3 supplements: wheat (W), sugar beet molasses (M), or sugar beet molasses mixed with sodium bicarbonate (Mbic) as a buffer to prevent low rumen pH. All feed for the entire experiment was prepared once from the same batches of ingredients. After preparation, daily portions of the TMR were immediately vacuum-packed and frozen. Each portion was thawed at room temperature overnight before being fed *ad libitum* twice daily. The chemical composition of the diets is shown in Table 1. The daily feed intake was measured by the difference between the amount of supply and orts.

2.2. Measurement techniques

2.2.1. CO_2-technique

Breath samples from the heifers were continuously measured every 20 s for 3 days (1 day at a time for each diet) in the metabolic cage to analyze the concentrations (parts per million) of CH_4 and carbon dioxide (CO_2). A portable continuous gas analyzer GASMET DX-4030 (Gasmet Technologies Oy, Helsinki, Finland) was used to analyze the breath concentrations based on Fourier Transformed Infrared (FTIR) detection. The metabolic cages were placed in a restricted ventilated barn which was kept open during the day time. The gas sampling inlet was attached to the metabolic cage, at the nose level of the heifers. The recorded concentrations of breath samples were stored in a data logger on a computer. Baseline barn air concentration was measured for 10 min during each experimental day. Measurements of CH_4 and CO_2 were taken in the metabolic cage continuously for 22 h for each animal, after which the heifers were moved to the respiration chamber for a similar time for the measurement of CO_2 emissions as described in the section below.

Table 1
Dietary and chemical composition of 3 diets.

Item	W	M	Mbic
Composition of the ration, g/kg DM			
Grass-clover silage	494	494	490
Wheat	353		
Sugar beet molasses		353	350
$NaHCO_3$			9.3
Soybean meal	141	141	140
Mineral and vitamins	12	12	12
Chemical composition, g/kg DM			
Ash	60.6	97.5	103
Protein[1]	172	177	175
Fat	25.8	16.5	16.7
Starch	243	7.6	3.8
Sugar	34.2	241	238
NDF	318	280	277

W = diet with ground wheat; M = diet with sugar beet molasses; MBic = diet with sugar beet molasses and sodium bicarbonate.
[1] Feedstuff table: composition and feeding value of feedstuffs for cattle Report No. 91, Danish Agricultural Advisory Service, 2000, English version.

2.2.2. Respiration chamber technique

The individual respiration measurements were performed for the measurement of total CO_2 in an open-air-circuit respiration chamber immediately after the metabolic cage measurements. Construction and function of the respirations chambers was described by Chwalibog et al. (2004). The animals had free access to the same diet in the chamber as it was in the metabolic cage and water was made available for 24 h. The climate in the chambers was kept constant at a temperature of 20 °C and a relative humidity of 60%. Chamber was calibrated by injecting know concentration of pure CO_2 and N_2 at the beginning of each measurement. The results obtained from calibrations indicate a high accuracy with an overall error of less than 1%. The concentrations of O_2, CO_2 and CH_4, temperature, relative humidity and rate of flow from the chamber were recorded automatically every 5 min. The exhaled CO_2 concentration was determined by the difference between the concentration of that in air-in and air-out. Data from the 22-h gas exchange measurements (for each diet) in the chamber was used as 2 h of the day were used to change animals.

2.3. Calculations

For the calculation of CH_4:CO_2 ratio from the breath samples, the average barn concentrations of CO_2 (705 ± 88.3 ppm) and CH_4 (26 ± 10.3 ppm) (means ± SD) were subtracted from the exhaled air concentrations to get the animal produced CO_2 and CH_4 concentrations. After correction, all values of corrected CO_2 below 400 ppm were removed in order to avoid the bias of samples containing a very low concentration of CH_4 and CO_2 generated when the animal's nose was not in the close proximity to the gas sampling inlet. The ratio of CH_4 to CO_2 was thereafter calculated.

Methane emission of the heifers was calculated from the breath sample analyses in 2 ways. Both calculations are based on the CH_4:CO_2 ratio measured in the metabolic cages and as described by Madsen et al. (2010), considering calculated total CO_2 calculated from heat production or measured total CO_2 in the respiration chamber (CO_2R). Heat production (HP) was calculated with animal parameters (metabolic weight, dairy weight gain, energy content of diet and days in pregnancy) as described in Eq. (1) by CIGR (2002). The amount of heat produced is necessary to know in order to calculate carbon dioxide production $CO_{2\ (HP)}$ according to the CO_2T as described by Pedersen et al. (2008) and shown in Eq. (2). The value $CO_{2\ (HP)}$ was used in the CO_2T calculated CH_4 production [Eq. (3)], and compared with calculated CH_4 produced based on CO_2 production measured in respiration chambers (CO_2R) [Eq. (4)]. The CH_4 (L/kg DMI) in the respiration chamber was calculated considering DMI from the previous day.

$$\mathrm{HP(watt)} = 7.64 \times \mathrm{BW}^{0.69} + Y\left[\frac{23}{M} - 1\right]\left[\frac{57.27 + 0.302 \times \mathrm{BW}}{1 - 0.171Y}\right] + 1.6 \times 10^{-5} \times P^3 \quad (1)$$

$$\mathrm{CO_{2\ (HP)}} = \mathrm{HPU} \times 180(\mathrm{L}) \times 24(\mathrm{h}) \quad (2)$$

$$\mathrm{CH_{4\ (HP)}} = \mathrm{CO_{2(HP)}} \times \frac{\mathrm{CH_4}}{\mathrm{CO_2}} \quad (3)$$

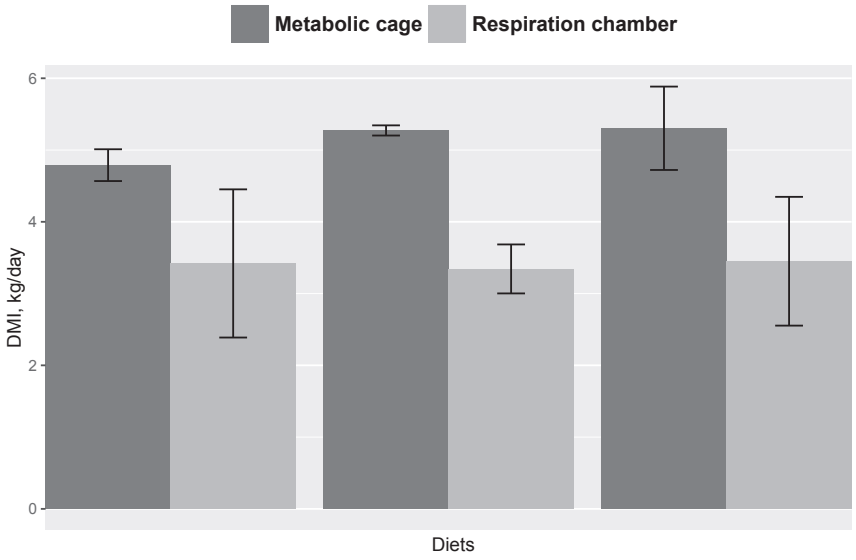

Fig. 1. Dry matter intake (DMI kg/day) of heifers fed 3 diets (W = wheat; M = molasses, and Mbic = molasses + sodium bicarbonate) in metabolic cage and respiration chamber. The bars indicate means ± SD of DMI (kg/day).

$$CH_{4(RC)} = CO_2R \times \frac{CH_4}{CO_2} \quad (4)$$

where HP = heat production of the animals; BW = body weight of the animals; Y = daily weight gain set as 0.5 kg/day; M = energy contents of the diet; P = days of pregnancy of the heifers; $CO_{2\,(HP)}$ = carbon dioxide production (L/day) calculated based on heat production; CO_2R = carbon dioxide production (L/day) measured in respiration chamber; HPU = heat producing unit calculated as HP/1000; $CH_{4\,(HP)}$ and $CH_{4\,(RC)}$ = methane calculated from $CO_{2\,(HP)}$ and $CO_{2\,(RC)}$; 180 = l of CO_2/HPU per hour; CH_4/CO_2 = measured CH_4:CO_2 ratio using the CO_2T breath sample analysis.

2.4. Statistical analysis

All statistical analyses were undertaken in the R statistical program (R Development Core Team, 2013). Daily carbon dioxide emission and DMI during the period of time the heifers were in the metabolic cages and in the respiration chamber were first analyzed as a response variable with a linear model considering diet and heifer as fixed variables. Thereafter, the differences in average hourly methane breath concentrations during 24 h were tested with a linear mixed model using the lmer function from the lme4 package (Bates and Sarkar, 2009). The R package lmer Test was used to compute P-values directly from the model (Kuznetsova et al., 2012). The primary model was fitted by maximum likelihood for BW, diet (3 levels) and DMI as fixed variables and the heifer identification as a random variable. The final model in Eq. (5) was selected by the stepwise elimination of the non-significant variables. The estimates of the responses were produced by fitting the final model with Restricted Maximum Likelihood (REML). The model was validated using an analysis of variance (ANOVA) based on the Akaike Information Criterion. The model residuals were checked for normality and homoscedasticity by visual inspection of qq-plots.

$$y_{ij} = \mu + \alpha_i + X\beta_{ij} + \delta_j + \varepsilon_{ij} \quad (5)$$

where y_{ij} is the response variable, $y = CH_4$ in L/day and L/kg DMI of diet i and heifer j, μ = overall mean, α_i = diet (W, M and Mbic), $X\beta_{ij}$ = DMI of heifer j (j is 1 to 3) for diet i, δ_j = random effect of heifer and ε_{ij} is the model residuals.

3. Results

Dry matter intake (kg/day) in the metabolic cage was not different ($P > 0.1$) during the 3 measurement periods. Similarly, no difference of the DMI (kg/day) was observed in the respiration chamber during the measurement periods. However, the DMI (kg/day) was significantly higher ($P < 0.001$) in the metabolic cage compared with the intake in the chamber (Fig. 1). The CH_4 estimations for 2 methods are presented in Table 2. All 3 diets showed that daily CH_4 (L/kg DMI) emissions estimated by CO_2T were of the same scale for the CO_2R. The measured CO_2 production in the respiration chamber (1,784 ± 193.5 L/day; means ± SD) was not different ($P = 0.39$) from the calculated CO_2 production (1,709 ± 52.1 L/day; means ± SD) using the CO_2T method (Fig. 2). The calculated CO_2 (L/day) using the CO_2T technique was positively correlated with the measured CO_2 (L/day) in the respiration chamber (Fig. 3) according to the body mass of the animal.

4. Discussion

4.1. Method comparison

The respiration chamber is the reference method for animal metabolism studies and total gas emissions, including CH_4. The CO_2T is a newly developed technique which uses the CH_4:CO_2 ratio from breath sample analysis of the animals to calculate CH_4

Table 2
Methane production of heifers fed 3 different diets, estimated using different methods.

Method	Diets	CH_4, L/day	CH_4, L/kg DMI
CO_2T	W	126.7[a]	25.1[a]
	M	144.8[b]	28.2[b]
	Mbic	154.0[c]	30.2[c]
CO_2R	W	142.9[a]	28.0[a]
	M	148.6[a]	29.0[a]
	Mbic	151.5[b]	29.8[b]

CH_4 = methane; DMI = dry matter intake; CO_2T = CO_2-method; CO_2R = CO_2 measured in respiration chamber; W = wheat; M = molasses; Mbic = molasses + sodium bicarbonate.
[a,b,c] Values in the same column with different superscripts indicate differences ($P < 0.05$) between diets for each method.

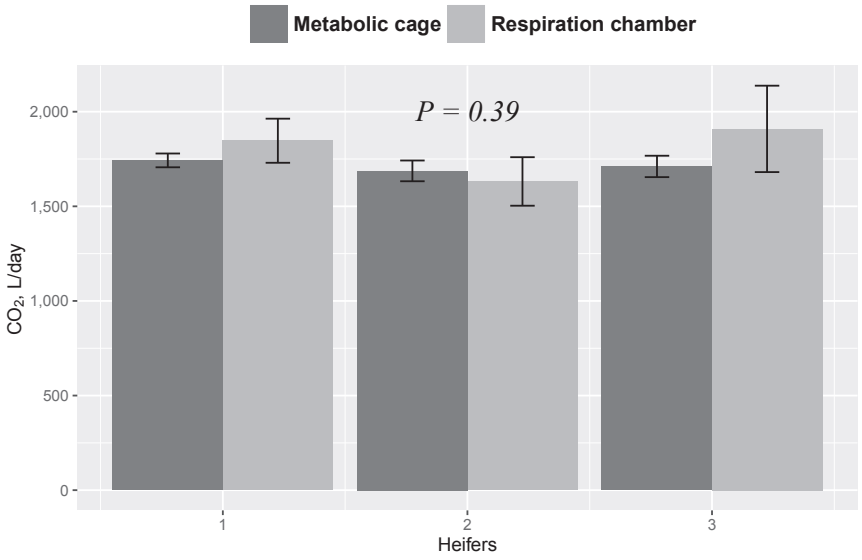

Fig. 2. Calculated total CO_2 (L/day) according to CO_2T vs. measured CO_2 (L/day) by respiration chamber. The bars indicate means ± SD of CO_2 (L/day) production. The *P*-value is the model probability for significant difference of CO_2 (L/day) production between 2 measurement techniques.

production. The majority of CH_4 produced in the rumen is emitted through the eructation (Place and Mitloehner, 2010). The maximum CH_4 emitted from the hind gut of dairy cows is reported to be 13% of total daily methane emission (Ellis et al., 2008). Therefore, the CO_2T method is valid in that the majority of the emission will be collected through breath sample analysis. The present results showed a lower DMI in the respiration chamber, in agreement with the previous study byPinares-Patino and Clark (2008), who also reported lower intake in the respiration chamber. Dry matter intake has a large influence on the daily mean CH_4 emission (Boadi et al., 2004). Thorbek (1980) found that animals fed *ad libitum* in the barn showed a significantly lower intake when moved into the chamber. In the same study, animals had a higher intake in the respiration chamber when fed restricted in the barn. The DMI appears to be reduced in the traditional steel box respiration chamber which completely isolates the animals from others. Reduction of DMI may be less when dairy cows are placed in a modern designed plexiglass respiration chamber, as was done by Hellwing et al. (2012).

The CH_4 production per unit of DMI was comparable among all of the methods in the present study. The CH_4 (L/kg DMI) estimated by the CO_2T was similar to the estimates by the CO_2R. This is presumed to be due to the fact that CO_2 produced in the chamber is not influenced by the one day lower DMI when in the chamber. The number of animals used in this study for the different methods was limited. Estimation of methane using the CO_2T could be undertaken in a commercial farm situation where large number of animals could be considered and the animals have a more natural behavior (Haque et al., 2014a, 2015). A recent study indicated that the total

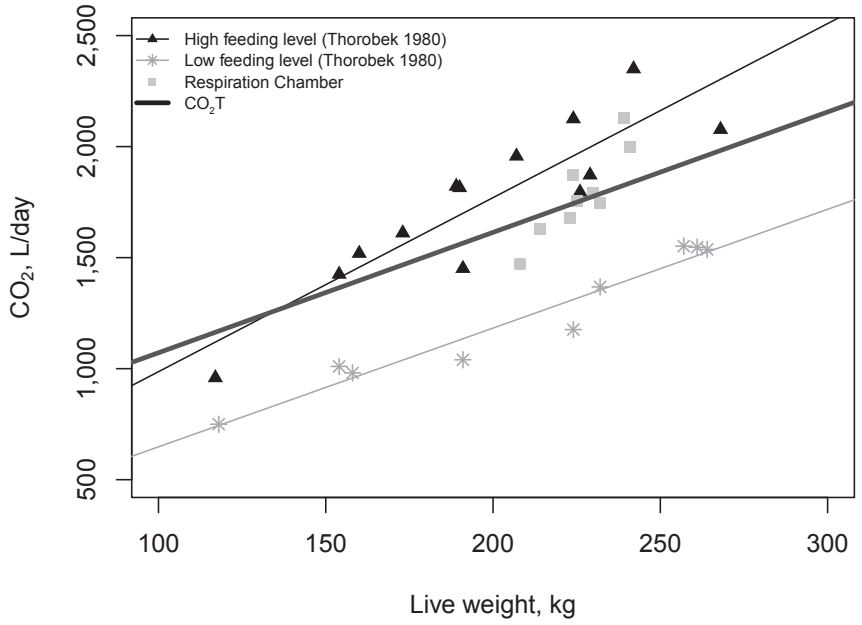

Fig. 3. Calculated and measured CO_2 (L/day) production from the heifers obtained by the CO_2 method (CO_2T) and respiration chamber (CO_2R) and compared with the previous results from respiration chamber study with growing bull calves at low and high feeding levels (Thorbek, 1980).

CO_2 concentration measured by the CO_2T varies with variable muzzle movement, muzzle position and possible air mix or cross contamination (Huhtanen et al., 2015), which ultimately affects CH_4 estimation. In this study, cross contamination was avoided by specific data filtering system as described in section 2.3. Use of muzzle sensor in the sampling inlet would be a further development of the measurement of CH_4 and CO_2 concentration by the CO_2T. We assume that the precision of the methane CO_2T estimates can be improved in this situation, either by measuring emissions from a large number of animals or measuring for a longer time without altering the natural movement of the animals.

4.2. Calculation of carbon dioxide production for methane estimation

The calculation of CO_2 production in the CO_2T is based on the results from metabolism experiments reported in the last several decades. The total CO_2 production of animals can be calculated using body mass, growth and production information or using the nutrients intake and utilization. The CO_2 production of animals is determined by the type of diet and nutrient concentration, levels of intake and body activity, which is closely related to metabolism or heat production of animals (CIGR). The accuracy of CH_4 estimation using CO_2T depends on the accuracy of calculated total CO_2 production (Madsen et al., 2014). The calculated CO_2 (by the CO_2T) and measured CO_2 in the respiration chamber (CO_2R) in this study showed a strong correlation ($r = 0.85$) with an average of 1,754 L/day and a deviation between the techniques of ±53 L/day. Moreover, Fig. 3 shows the CO_2 produced by bull calves fed either high or low feeding level (Thorbek, 1980)were respectively higher and lower than either the CO_2T or CO_2R estimations. Therefore, it can be concluded that the CO_2T can predict the total CO_2 production with a reasonable accuracy because this prediction is comparable to the reference method i.e., respiration chambers. According to the CO_2T, CO_2 emission is multiplied with the CH_4:CO_2 ratio from breath sample analysis to calculate the daily CH_4 emission (Haque et al., 2014a, 2014b). The CH_4 estimation can therefore be influenced by the total CO_2 production as well as variation in the CH_4:CO_2 ratio (Haque et al., 2015). Bjerg et al. (2012) found diurnal variation of the CH_4:CO_2 ratio, which will influence the CH_4 estimation. This diurnal variation was considered in the present study by analyzing breath samples over 22 h. From the comparative values of CH_4 (L/kg DMI) estimated by the CO_2T, and CO_2R, it can be seen that the CO_2 T estimated CH_4 emissions with reasonable accuracy and precision.

5. Conclusions

The results show that the DMI was less in the respiration chamber than in the metabolic cages. All 3 diets showed a similar scale of methane estimation by the CO_2T and CO_2R. The variation between estimated CO_2 productions was within the acceptable range for the 2 techniques (CO_2T and CO_2R). The CO_2T can predict CH_4 emissions with a reasonable accuracy and precision as compared with the chamber technique. The precision can be improved either by using more animals or longer measurement period.

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References

Bates DM, Sarkar D. lme4: linear mixed-effects models using S4 classes, R package version 0.99875-6. 2009. http://cran.r-project.org/web/packages/lme4/index.html [accessed 17.04.13.].
Benchaar C, Greathead H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim Feed Sci Technol 2011;166–167:338–55.
Bjerg B, Zhang GQ, Madsen J, Rom HB. Methane emission from naturally ventilated livestock buildings can be determined from gas concentration measurements. Environ Monit Assess 2012;184(10):5989–6000.
Boadi DA, Wittenberg KM, Scott SL, Burton D, Buckley K, Small JA, et al. Effect of low and high forage diet on enteric and manure pack greenhouse gas emissions from a feedlot. Can J Anim Sci 2004;84(3):445–53.
Chwalibog A, Tauson AH, Thorbek G. Energy metabolism and substrate oxidation in pigs during feeding, starvation and re-feeding. J Anim Physiol Anim Nutr (Berl) 2004;88(3–4):101–12.
CIGR. Climatization of animal houses – heat and moisture production at animal and house level, Danish Institute of Agricultural Sciences, P.O Box 536, DK-8700 Horsens, Denmark. 2002. p. 1–46.
Ellis JL, Dijkstra J, Kebreab E, Bannink A, Odongo NE, McBride BW, et al. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J Agric Sci 2008;146(Special Issue 02):213–33.
Friggens NC, Oldham JD, Dewhurst RJ, Horgan G. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. J Dairy Sci 1998;81(5):1331–44.
Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, et al. Tackling climate change through livestock – a global assessment of emissions and mitigation opportunities. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO); 2013.
Haque MN, Cornou C, Madsen J. Estimation of methane emission using the CO_2 method from dairy cows fed concentrate with different carbohydrate compositions in automatic milking system. Livest Sci 2014a;164(0):57–66.
Haque MN, Roggenbuck M, Khanal P, Nielsen MO, Madsen J. Development of methane emission from lambs fed milk replacer and cream for a prolonged period. Anim Feed Sci Technol 2014b;198(0):38–48.
Haque MN, Cornou C, Madsen J. Individual variation and repeatability of methane production from dairy cows estimated by the CO_2 method in automatic milking system. Animal 2015;9(9):1567–76.
Hellwing ALF, Brask M, Lund P, Weisbjerg MR. Effect of carbohydrate source and rumen pH on enteric methane from dairy cows. In: Proc. international symposium on emission of gas and dust from livestock (EMILI 2012), Saint-Malo, France; 2012. p. 206–8.
Hindrichsen IK, Wettstein HR, Machmüller A, Soliva CR, Bach Knudsen KE, Madsen J, et al. Effects of feed carbohydrates with contrasting properties on rumen fermentation and methane release in vitro. Can J Anim Sci 2004;84(2):265–76.
Huhtanen P, Cabezas-Garcia EH, Utsumi S, Zimmerman S. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J Dairy Sci 2015;98(5):3394–409.
Johnson KA, Johnson DE. Methane emissions from cattle. J Anim Sci 1995;73(8):2483–92.
Kuznetsova A, Brockhoff PB, Christensen RHB. Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). 2012. http://cran.r-project.org/web/packages/lmerTest/index.html [accessed 27.04.13.].
Madsen J, Bjerg BS, Hvelplund T, Weisbjerg MR, Lund P. Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants. Livest Sci 2010;129(1–3):223–7.
Madsen J, Chwalibogb A, Hvelplundc T, Weisbjergc MR. Use of carbon dioxide as a breath marker. In: Proc. book of abstracts of 65th annual meeting of the European Federation of Animal Science, Copenhagen, Denmark; 2014. p. 408.
Moss AR, Jouany JP, Newbold J. Methane production by ruminants: its contribution to global warming. Ann Zootech 2000;49(3):231–53.
Pedersen S, Blanes-Vidal V, Joergensen H, Chwalibog A, Haeussermann A, Heetkamp MJW, et al. Carbon dioxide production in animal houses: a literature review. Agricultural Engineering International: CIGR Ejournal; 2008. http://www.cigrjournal.org/index.php/Ejounral/article/viewFile/1205/1132 [accessed 07.07.14.].
Pinares-Patino CS, Clark H. Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. Aust J Exp Agric 2008;48(1–2):223–9.
Place SE, Mitloehner FM. Invited review: contemporary environmental issues: a review of the dairy industry's role in climate change and air quality and the potential of mitigation through improved production efficiency. J Dairy Sci 2010;93(8):3407–16.
R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. http://www.R-project.org [accessed 17.04.13.].
Storm IMLD, Hellwing ALF, Nielsen NI, Madsen J. Methods for measuring and estimating methane emission from ruminants. Animals 2012;2(4):160–83.
Thorbek G. Studies on protein and energy metabolism in growing calves. Copenhagen, Denmark: Landhusholdingsselskabets forlag; 1980. p. 1–105.